



# SEQUENCE LISTING

<110> Bron, Sierd  
Jongbloed, Jan D.H.  
Mueller, Joerg P.  
Van Dijl, Jan M.

<120> Twin-Arginine Translocation in Bacillus

<130> GC634-2

<140> US 09/954,737

<141> 2001-09-17

<150> US 60/233,610

<151> 2000-09-18

<160> 86

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<212> PRT

<213> Escherichia coli

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<213> *Bacillus subtilis*

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Ala Lys Arg Thr Leu Leu Glu Phe Lys Ser Ala Thr Lys Ser Leu Val
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<213> *Bacillus subtilis*

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Lys Ala Leu Ser Glu Phe Lys Gln Ala Thr Ser Gly Leu Thr Gln Asp
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<213> *Escherichia coli*

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Lys	Val	Glu	Lys	Ala	Ser	Leu	Thr	Asn	Leu	Thr	Pro	Glu	Leu	Lys	Ala
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<213> Escherichia coli

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Ile	Leu	Ser	Ala	Pro	Val	Ile	Leu	Tyr	Gln	Val	Trp	Ala	Phe	Ile	Ala
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Pro	Ala	Leu	Tyr	Lys	His	Glu	Arg	Arg	Leu	Val	Val	Pro	Leu	Leu	Val
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Leu	Phe	Glu	Ile	Gly	Val	Phe	Phe	Ser	Arg	Phe	Tyr	Val	Gly	Lys	Gly
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Glu Glu

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<213> Bacillus subtilis

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Phe Ile Ala Gly Phe Phe Leu Ala Lys Pro Ile Ile Val Tyr Leu Gln  
35 40 45  
Glu Thr Asp Glu Ala Lys Gln Leu Thr Leu Asn Ala Phe Asn Leu Thr  
50 55 60  
Asp Pro Leu Tyr Val Phe Met Gln Phe Ala Phe Ile Ile Gly Ile Val  
65 70 75 80  
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Gly Leu Tyr Glu Lys Glu Arg Lys Val Thr Leu Ser Tyr Ile Pro Val  
100 105 110  
Ser Ile Leu Leu Phe Leu Ala Gly Leu Ser Phe Ser Tyr Tyr Ile Leu  
115 120 125  
Phe Pro Phe Val Val Asp Phe Met Lys Arg Ile Ser Gln Asp Leu Asn  
130 135 140  
Val Asn Gln Val Ile Gly Ile Asn Glu Tyr Phe His Phe Leu Leu Gln  
145 150 155 160  
Leu Thr Ile Pro Phe Gly Leu Leu Phe Gln Met Pro Val Ile Leu Met  
165 170 175  
Phe Leu Thr Arg Leu Gly Ile Val Thr Pro Met Phe Leu Ala Lys Ile  
180 185 190  
Arg Lys Tyr Ala Tyr Phe Thr Leu Leu Val Ile Ala Ala Leu Ile Thr  
195 200 205  
Pro Pro Glu Leu Leu Ser His Met Met Val Thr Val Pro Leu Leu Ile  
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Ala Phe Leu Phe Val Gln Asp Ile Tyr Asp Trp Leu Ile Arg Asp Leu  
35 40 45  
Asp Gly Lys Leu Ala Val Leu Gly Pro Ser Glu Ile Leu Trp Val Tyr  
50 55 60

Met Met Leu Ser Gly Ile Cys Ala Ile Ala Ala Ser Ile Pro Val Ala  
 65 70 75 80  
 Ala Tyr Gln Leu Trp Arg Phe Val Ala Pro Ala Leu Thr Lys Thr Glu  
 85 90 95  
  
 Arg Lys Val Thr Ile Met Tyr Ile Met Tyr Ile Pro Gly Leu Phe Ala  
 100 105 110  
 Leu Phe Leu Ala Gly Ile Ser Phe Gly Tyr Phe Val Leu Phe Pro Ile  
 115 120 125  
 Val Leu Ser Phe Leu Thr His Leu Ser Ser Gly His Phe Glu Thr Met  
 130 135 140  
 Phe Thr Ala Asp Arg Tyr Phe Arg Phe Met Val Asn Leu Ser Leu Pro  
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 Phe Gly Phe Leu Phe Glu Met Pro Leu Val Val Met Phe Leu Thr Arg  
 165 170 175  
 Leu Gly Ile Leu Asn Pro Tyr Arg Leu Ala Lys Ala Arg Lys Leu Ser  
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 Tyr Phe Leu Leu Ile Val Val Ser Ile Leu Ile Thr Pro Pro Asp Phe  
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 35 40 45  
 Ile Val Ala Leu Ile Gly Gly Phe Phe Leu Ala Val Pro Val Ile Thr  
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 Phe Leu Gln Asn Ser Pro Gln Ala Ala Asp Met Pro Phe Asn Ala Phe  
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Ala	Leu	Val	Leu	Ile	Ile	Pro	Val	Ile	Leu	Tyr	Gln	Leu	Trp	Ala	Phe
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Leu	Phe	Gln	Leu	Thr	Ile	Pro	Phe	Gly	Leu	Leu	Phe	Gln	Leu	Pro	Val
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Val	Val	Met	Phe	Leu	Thr	Arg	Leu	Gly	Val	Val	Thr	Pro	Thr	Phe	Leu
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225					230					235					240
Met	Leu	Ile	Leu	Tyr	Glu	Ile	Ser	Ile	Thr	Ile	Ser	Ala	Ile	Thr	Tyr
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Leu	Ile	Phe	Ile	Ala	Ile	Ala	Ile	Leu	Phe						
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subtilis

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Met	Val	Ser	Ile	Arg	Arg	Ser	Phe	Glu	Ala	Tyr	Val	Asp	Asp	Met	Asn
1				5				10						15	
Ile	Ile	Thr	Val	Leu	Ile	Pro	Ala	Glu	Gln	Lys	Glu	Ile	Met		
			20					25					30		

<210> 54

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> predicted twin-arginine signal peptides of B.  
subtilis

<400> 54

Met	Ala	Ala	Tyr	Ile	Ile	Arg	Arg	Thr	Leu	Met	Ser	Ile	Pro	Ile	Leu
1				5				10						15	
Leu	Gly	Ile	Thr	Ile	Leu	Ser	Phe	Val	Ile	Met	Lys	Ala	Ala	Pro	Gly
			20					25					30		

<210> 55

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> predicted twin-arginine signal peptides of B.  
subtilis

<400> 55

Met	Lys	Phe	Val	Lys	Arg	Arg	Ile	Ile	Ala	Leu	Val	Thr	Ile	Leu	Met
1				5					10					15	

Leu Ser Val Thr Ser Leu Phe Ala Leu Gln Pro Ser Ala Lys Ala Ala  
                   20                  25                  30  
 Glu His

<210> 56  
 <211> 32  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> predicted twin-arginine signal peptides of B.  
           subtilis

<400> 56  
 Met Leu Lys Tyr Ile Gly Arg Arg Leu Val Tyr Met Ile Ile Thr Leu  
   1                  5                  10                  15  
 Phe Val Ile Val Thr Val Thr Phe Phe Leu Met Gln Ala Ala Pro Gly  
                   20                  25                  30

<210> 57  
 <211> 42  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> predicted twin-arginine signal peptides of B.  
           subtilis

<400> 57  
 Met Thr Ser Pro Thr Arg Arg Arg Thr Ala Lys Arg Arg Arg Arg Lys  
   1                  5                  10                  15  
 Leu Asn Lys Arg Gly Lys Leu Leu Phe Gly Leu Leu Ala Val Met Val  
                   20                  25                  30  
 Cys Ile Thr Ile Trp Asn Ala Leu His Arg  
           35                  40

<210> 58  
 <211> 54  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> predicted twin-arginine signal peptides of B.  
           subtilis

<400> 58  
 Met Ala Tyr Asp Ser Arg Phe Asp Glu Trp Val Gln Lys Leu Lys Glu  
   1                  5                  10                  15  
 Glu Ser Phe Gln Asn Asn Thr Phe Asp Arg Arg Lys Phe Ile Gln Gly  
                   20                  25                  30  
 Ala Gly Lys Ile Ala Gly Leu Ser Leu Gly Leu Thr Ile Ala Gln Ser  
           35                  40                  45  
 Val Gly Ala Phe Glu Val  
           50

<210> 59  
 <211> 36



<212> PRT  
<213> Artificial Sequence

<220>

<223> predicted twin-arginine signal peptides of B.  
subtilis

<400> 59

Met	Gly	Gly	Lys	His	Asp	Ile	Ser	Arg	Arg	Gln	Phe	Leu	Asn	Tyr	Thr
1				5				10					15		
Leu	Thr	Gly	Val	Gly	Gly	Phe	Met	Ala	Ala	Ser	Met	Leu	Met	Pro	Met
			20					25					30		
Val	Arg	Phe	Ala												
			35												

<210> 60

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> predicted twin-arginine signal peptides of B.  
subtilis

<400> 60

Met	Leu	Leu	Lys	Arg	Arg	Ile	Gly	Leu	Leu	Leu	Ser	Met	Val	Gly	Val
1				5				10						15	
Phe	Met	Leu	Leu	Ala	Gly	Cys	Ser	Ser	Val						
			20					25							

<210> 61

<211> 39

<212> PRT

<213> Artificial Sequence

<220>

<223> predicted twin-arginine signal peptides of B.  
subtilis

<400> 61

Met	Lys	Lys	Thr	Leu	Thr	Thr	Ile	Arg	Arg	Ser	Ser	Ile	Ala	Arg	Arg
1				5				10					15		
Leu	Ile	Ile	Ser	Phe	Leu	Leu	Ile	Leu	Ile	Val	Pro	Ile	Thr	Ala	Leu
			20					25					30		
Ser	Val	Ser	Ala	Tyr	Gln	Ser									
			35												

<210> 62

<211> 35

<212> PRT

<213> Artificial Sequence

<220>

<223> predicted twin-arginine signal peptides of B.  
subtilis

<400> 62

Met Lys Lys Arg Lys Arg Arg Asn Phe Lys Arg Phe Ile Ala Ala Phe  
 1 5 10 15  
 Leu Val Leu Ala Leu Met Ile Ser Leu Val Pro Ala Asp Val Leu Ala  
 20 25 30  
 Lys Ser Thr  
 35

<210> 63  
 <211> 33  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> predicted twin-arginine signal peptides of B.  
 subtilis

<400> 63  
 Lys Arg Arg Lys Phe Ser Ser Val Val Ala Ala Val Leu Ile Phe Ala  
 1 5 10 15  
 Leu Ile Phe Ser Leu Phe Ser Pro Gly Thr Lys Ala Ala Ala Ala Gly  
 20 25 30  
 Ala

<210> 64  
 <211> 35  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> predicted twin-arginine signal peptides of B.  
 subtilis

<400> 64  
 Met Glu Met Phe Asp Leu Glu Phe Met Arg Arg Ala Phe Leu Ala Gly  
 1 5 10 15  
 Gly Met Ile Ala Val Met Ala Pro Ile Leu Gly Val Tyr Leu Val Leu  
 20 25 30  
 Arg Arg Gln  
 35

<210> 65  
 <211> 26  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> predicted twin-arginine signal peptides of B.  
 subtilis

<400> 65  
 Met Lys Lys Arg Arg Lys Ile Cys Tyr Cys Asn Thr Ala Leu Leu Leu  
 1 5 10 15  
 Met Ile Leu Leu Ala Gly Cys Thr Asp Ser  
 20 25

<210> 66  
 <211> 20

<212> PRT  
<213> Artificial Sequence

<220>

<223> predicted twin-arginine signal peptides of B.  
subtilis

<400> 66

Met Arg Arg Ile Leu Ser Ile Leu Val Phe Ala Ile Met Leu Ala Gly  
1 5 10 15  
Cys Ser Ser Asn

20

<210> 67

<211> 43

<212> PRT

<213> Artificial Sequence

<220>

<223> predicted twin-arginine signal peptides of B.  
subtilis

<400> 67

Met Ser Ala Gly Lys Ser Tyr Arg Lys Lys Met Lys Gln Arg Arg Met  
1 5 10 15  
Asn Met Lys Ile Ser Lys Tyr Ala Leu Gly Ile Leu Met Leu Ser Leu  
20 25 30  
Val Phe Val Leu Ser Ala Cys Gly Asn Asn Asn  
35 40

<210> 68

<211> 42

<212> PRT

<213> Artificial Sequence

<220>

<223> predicted twin-arginine signal peptides of B.  
subtilis

<400> 68

Lys Lys Arg Val Ala Gly Trp Tyr Arg Arg Met Lys Ile Lys Asp Lys  
1 5 10 15  
Leu Phe Val Phe Leu Ser Leu Ile Met Ala Val Ser Phe Leu Phe Val  
20 25 30  
Tyr Ser Gly Val Gln Tyr Ala Phe His Val  
35 40

<210> 69

<211> 38

<212> PRT

<213> Artificial Sequence

<220>

<223> predicted twin-arginine signal peptides of B.  
subtilis

<400> 69

Met Arg Arg Ser Cys Leu Met Ile Arg Arg Arg Lys Arg Met Phe Thr  
1 5 10 15  
Ala Val Thr Leu Leu Val Leu Leu Val Met Gly Thr Ser Val Cys Pro  
20 25 30  
Val Lys Ala Glu Gly Ala  
35

<210> 70

<211> 38

<212> PRT

<213> Artificial Sequence

<220>

<223> predicted twin-arginine signal peptides of B.  
subtilis

<400> 70

Met Arg Ile Gln Lys Arg Arg Thr His Val Glu Asn Ile Leu Arg Ile  
1 5 10 15  
Leu Leu Pro Pro Ile Met Ile Leu Ser Leu Ile Leu Pro Thr Pro Pro  
20 25 30  
Ile His Ala Glu Glu Ser  
35

<210> 71

<211> 32

<212> PRT

<213> Artificial Sequence

<220>

<223> predicted twin-arginine signal peptides of B.  
subtilis

<400> 71

Met Leu Arg Asp Leu Gly Arg Arg Val Val Ala Ile Ala Ala Ile Leu  
1 5 10 15  
Ser Gly Ile Ile Leu Gly Gly Met Ser Ile Ser Leu Ala Asn Met Pro  
20 25 30

<210> 72

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> predicted twin-arginine signal peptides of B.  
subtilis

<400> 72

Met Lys Lys Met Ser Arg Arg Gln Phe Leu Lys Gly Met Phe Gly Ala  
1 5 10 15  
Leu Ala Ala Gly Ala Leu Thr Ala Gly Gly Gly Tyr Gly Tyr Ala Arg  
20 25 30  
Tyr Leu

<210> 73  
 <211> 28  
 <212> PRT  
 <213> Artificial Sequence  
  
 <220>  
 <223> predicted twin-arginine signal peptides of B.  
           subtilis  
  
 <400> 73  
 Met Arg Arg Phe Leu Leu Asn Val Ile Leu Val Leu Ala Ile Val Leu  
   1                  5                  10                  15  
 Phe Leu Arg Tyr Val His Tyr Ser Leu Glu Pro Glu  
           20                  25  
  
 <210> 74  
 <211> 29  
 <212> PRT  
 <213> Artificial Sequence  
  
 <220>  
 <223> predicted twin-arginine signal peptides of B.  
           subtilis  
  
 <400> 74  
 Met Phe Glu Ser Glu Ala Glu Leu Arg Arg Ile Arg Ile Ala Leu Val  
   1                  5                  10                  15  
 Trp Ile Ala Val Phe Leu Leu Phe Gly Ala Cys Gly Asn  
           20                  25  
  
 <210> 75  
 <211> 37  
 <212> PRT  
 <213> Artificial Sequence  
  
 <220>  
 <223> predicted twin-arginine signal peptides of B.  
           subtilis  
  
 <400> 75  
 Met Gln Lys Tyr Arg Arg Arg Asn Thr Val Ala Phe Thr Val Leu Ala  
   1                  5                  10                  15  
 Tyr Phe Thr Phe Phe Ala Gly Val Phe Leu Phe Ser Ile Gly Leu Tyr  
           20                  25                  30  
 Asn Ala Asp Asn Leu  
           35  
  
 <210> 76  
 <211> 34  
 <212> PRT  
 <213> Artificial Sequence  
  
 <220>  
 <223> predicted twin-arginine signal peptides of B.  
           subtilis  
  
 <400> 76

Met Met Leu Asn Met Ile Arg Arg Leu Leu Met Thr Cys Leu Phe Leu  
 1 5 10 15  
 Leu Ala Phe Gly Thr Thr Phe Leu Ser Val Ser Gly Ile Glu Ala Lys  
 20 25 30  
 Asp Leu

<210> 77  
 <211> 44  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> predicted twin-arginine signal peptides of B.  
 subtilis

<400> 77  
 Met Ala Glu Arg Val Arg Val Arg Val Arg Lys Lys Lys Lys Ser Lys  
 1 5 10 15  
 Arg Arg Lys Ile Leu Lys Arg Ile Met Leu Leu Phe Ala Leu Ala Leu  
 20 25 30  
 Leu Val Val Val Gly Leu Gly Gly Tyr Lys Leu Tyr  
 35 40

<210> 78  
 <211> 47  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> predicted twin-arginine signal peptides of B.  
 subtilis

<400> 78  
 Met Ser Asp Glu Gln Lys Lys Pro Glu Gln Ile His Arg Arg Asp Ile  
 1 5 10 15  
 Leu Lys Trp Gly Ala Met Ala Gly Ala Ala Val Ala Ile Gly Ala Ser  
 20 25 30  
 Gly Leu Gly Gly Leu Ala Pro Leu Val Gln Thr Ala Ala Lys Pro  
 35 40 45

<210> 79  
 <211> 54  
 <212> PRT  
 <213> Bacillus subtilis

<400> 79  
 Met Ala Tyr Asp Ser Arg Phe Asp Glu Trp Val Gln Lys Leu Lys Glu  
 1 5 10 15  
 Glu Ser Phe Gln Asn Asn Arg Phe Asp Arg Arg Lys Phe Ile Gln Gly  
 20 25 30  
 Ala Gly Lys Ile Ala Gly Leu Ser Leu Gly Leu Thr Ile Ala Gln Ser  
 35 40 45  
 Val Gly Ala Phe Glu Val  
 50

<210> 80  
 <211> 65

<212> PRT

<213> Streptomyces coelicolor

<400> 80

```
Met Thr Pro Ala Asn His Gln Ala Pro Thr Ser Ala Pro Ser Pro Ala
 1          5          10          15
Pro Ser Gln Ser Ser His Ala Pro Glu Leu Arg Ala Ala Ala Arg Ser
      20          25          30
Leu Gly Arg Arg Arg Phe Leu Thr Val Thr Gly Ala Ala Ala Leu
      35          40          45
Ala Phe Ala Val Asn Leu Pro Ala Ala Gly Thr Ala Ser Ala Ala Glu
      50          55          60
Leu
65
```

<210> 81

<211> 60

<212> PRT

<213> Streptomyces coelicolor

<400> 81

```
Met Ala Pro Thr Gly Arg Pro Ser Ala Leu Ala Glu His Ala Phe Ser
 1          5          10          15
Pro His Asp Ala Val Leu Gly Ala Ala Ala Arg His Leu Gly Arg Arg
      20          25          30
Arg Phe Leu Thr Val Thr Ala Ala Ala Ala Leu Ala Phe Ser Thr
      35          40          45
Asn Leu Pro Ala Arg Gly Ala Val Ala Ala Pro Glu
      50          55          60
```

<210> 82

<211> 47

<212> PRT

<213> Streptomyces coelicolor

<400> 82

```
Met Thr Ser Arg His Arg Ala Ser Glu Asn Ser Arg Thr Pro Ser Arg
 1          5          10          15
Arg Thr Val Val Lys Ala Ala Ala Ala Gly Ala Val Leu Ala Ala Pro
      20          25          30
Leu Ala Ala Ala Leu Pro Ala Gly Ala Ala Asp Ala Ala Pro Ala
      35          40          45
```

<210> 83

<211> 53

<212> PRT

<213> Streptomyces tendae

<400> 83

```
Met Thr Pro Ala Ala Arg Pro Ser Gln His Ala Pro Glu Leu Arg Ala
 1          5          10          15
Ala Ala Arg His Leu Gly Arg Arg Arg Phe Leu Thr Val Thr Gly Ala
      20          25          30
Ala Ala Ala Leu Ala Phe Ala Val Asn Leu Pro Ala Ala Gly Thr Ala
      35          40          45
Ala Ala Ala Glu Leu
      50
```

<210> 84  
 <211> 43  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> predicted twin-arginine signal peptides of B.  
 subtilis

<400> 84  
 Met Ser Pro Ala Gln Arg Arg Ile Leu Leu Tyr Ile Leu Ser Phe Ile  
 1 5 10 15  
 Phe Val Ile Gly Ala Val Val Tyr Phe Val Lys Ser Asp Tyr Leu Phe  
 20 25 30  
 Thr Leu Ile Phe Ile Ala Ile Ala Ile Leu Phe  
 35 40

<210> 85  
 <211> 34  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> predicted twin-arginine signal peptides of B.  
 subtilis

<400> 85  
 Met Lys Arg Arg Lys Phe Ser Ser Val Val Ala Ala Val Leu Ile Phe  
 1 5 10 15  
 Ala Leu Ile Phe Ser Leu Phe Ser Pro Gly Thr Lys Ala Ala Ala Ala  
 20 25 30  
 Gly Ala

<210> 86  
 <211> 43  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> predicted twin-arginine signal peptides of B.  
 subtilis

<400> 86  
 Met Lys Lys Arg Val Ala Gly Trp Tyr Arg Arg Met Lys Ile Lys Asp  
 1 5 10 15  
 Lys Leu Phe Val Phe Leu Ser Leu Ile Met Ala Val Ser Phe Leu Phe  
 20 25 30  
 Val Tyr Ser Gly Val Gln Tyr Ala Phe His Val  
 35 40